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Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy

YVO W. SIJPKENS, SIMONE A. JOOSTEN, MAN-CHI WONG, FRIEDO W. DEKKER, HALLGRIMUR BENEDIKTSSON, INGEBORG M. BAJEMA, JAN A. BRUIJN, and LEENDERT C. PAUL

Department of Nephrology, Department of Epidemiology and Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands; and Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada

Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy.

Background. Chronic transplant glomerulopathy is an uncommon cause of chronic transplant dysfunction of unknown pathogenesis. We evaluated the epidemiologic, clinical, and histologic features of chronic transplant glomerulopathy. To determine the possible contribution of humoral immune responses, we assessed glomerular deposition of C4d.

Methods. From a cohort of 1111 kidney transplants (1983 to 2001) with at least 6 months of graft function, we identified 18 cases with chronic transplant glomerulopathy (1.6%) showing double contours of the glomerular basement membrane (GBM) on light microscopy. To assess the risk factors, this group was compared with 739 patients with stable function using multivariate Cox regression analysis. Paraffin sections of 11/18 biopsies were stained with polyclonal C4d antibodies. Sera of 13/18 patients could be tested for antidonor human leukocyte antigen (HLA) antibodies by enzyme-linked immunosorbent assay (ELISA). Patients with chronic allograft nephropathy without chronic transplant glomerulopathy or predominant cyclosporine nephrotoxicity were used as controls.

Results. Chronic transplant glomerulopathy was diagnosed at a median of 8.3 (range 2.6–12.5) years posttransplantation. Panel reactive antibodies at time of transplantation, RR 1.23 (1.05–1.45) per 10% increase, and late acute rejection episodes, RR 7.6 (1.8–31.7), were independently associated with chronic transplant glomerulopathy. We found glomerular C4d deposits in 10/11 biopsies showing chronic transplant glomerulopathy and in only 2/13 controls. Peritubular capillary C4d deposits and donor-specific anti-HLA antibodies were demonstrated in 4 and 3 of the 10 patients with glomerular C4d deposits, respectively.

Conclusion. Presensitization and late acute rejection episodes were the risk factors identified. Glomerular C4d deposits suggest that chronic transplant glomerulopathy emerges from in situ humoral rejection. Chronic transplant glomerulopathy should be considered as a manifestation of immune-mediated injury.

Key words: kidney transplantation, chronic rejection, transplant glomerulopathy, risk factors, C4d.

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Chronic transplant glomerulopathy emerges in approximately 5% to 15% of transplants with chronic rejection [1–3], and is characterized by reduplication of the glomerular basement membrane (GBM) in the absence of de novo or recurrent glomerulonephritis [2]. Its extent is used to grade the severity of this entity in the Banff 97 classification [4]. Chronic transplant glomerulopathy usually occurs in the background of chronic allograft nephropathy (i.e., interstitial fibrosis and tubular atrophy with or without fibrous intimal thickening of arteries [4, 5]). Immunofluorescence microscopy is negative or shows mesangial granular deposits of IgM with greater intensity than C3 [1, 6]. Electron microscopy reveals reduplication of the GBM and subendothelial accumulation of electron-lucent material, distinguishing chronic transplant glomerulopathy from recurrent membranoproliferative glomerulonephritis [6]. Marked reduplication of peritubular capillary basement membranes is strongly associated with chronic transplant glomerulopathy [7]. Acute transplant glomerulitis, characterized by mononuclear cell infiltrate and endothelial cell enlargement, may precede chronic transplant glomerulopathy [1, 4, 8].

Recently, it has been suggested that allograft glomerulopathy should be separated from chronic rejection, as its pathogenesis is not understood [5]. Furthermore, a new classification of renal allograft rejection incorporates cellular and humoral mechanisms of injury [9, 10]. Chronic transplant glomerulopathy has been associated with circulating antidonor human leukocyte antigen (HLA) antibodies and the deposition of the complement split product C4d in peritubular capillaries, suggesting antibody-mediated injury [11, 12]. Regele et al [13] produced a polyclonal anti-C4d antibody that, in contrast to monoclonal antibodies, can be used on paraffin sections and does not stain normal glomeruli. However, glomerular deposits of C4d could be detected in only a minority of chronic transplant glomerulopathy biopsies [12].

The aim of the current study was to determine the incidence, risk factors, clinical characteristics and prognostic factors of chronic transplant glomerulopathy in

comparison with chronic allograft nephropathy without chronic transplant glomerulopathy. Furthermore, circulating antidonor HLA antibodies and glomerular deposition of C4d were assessed to determine whether humoral immunity is involved in the development of chronic transplant glomerulopathy.

METHODS

Patients

All 1111 cadaveric ($N = 832$), living related kidney ($N = 163$), and simultaneous kidney pancreas ($N = 116$) transplants done at the Leiden University Medical Center between January 1983 and January 2001 with at least 6 months of graft function were reviewed. There were 168 repeat transplants. Patients were followed to graft loss, death, or January 1, 2002. The initial immunosuppressive regimen consisted of prednisone and cyclosporine and/or azathioprine. In 1996, Sandimmune was changed to Neoral and mycophenolate mofetil was added as a third baseline drug.

Histopathology

We reviewed the biopsies that were taken beyond 6 months posttransplantation for clinical indications, including declining renal function or significant proteinuria. Recurrent ($N = 56$) or de-novo glomerulonephritis ($N = 11$) and predominant cyclosporine toxicity ($N = 53$) were excluded by clinical and histologic means. Cyclosporine nephrotoxicity was characterized by arteriolar hyalinosis and stabilization of renal function after dose reduction or switch to mycophenolate [14].

In 130 cases the histology indicated a diagnosis of chronic allograft nephropathy. All biopsies were reexamined and if transplant glomerulopathy was present, sections were scored according to the Banff 97 schema [4] by a single pathologist (H.B.). Eighteen patients met the definition of chronic transplant glomerulopathy according to the following criteria: (*1*) light microscopy showing "double contours" of the GBM in at least 10% of the most severely affected tuft [4]; (*2*) immunofluorescence negative or showing scant depositions of IgM with greater intensity than C3 [6]; and (*3*) recipient's original renal disease other than membranoproliferative glomerulonephritis and absence of hepatitis C seropositivity at time of transplantation [6, 15]. Unfortunately, no material was available to perform electron microscopy on these biopsies.

Four patients had acute transplant glomerulitis defined by mononuclear cell infiltration and endothelial cell enlargement in the absence of double contours of the GBM [4, 16]. Finally, 108 patients had chronic allograft nephropathy without predominant clinical and histologic signs of transplant glomerulopathy, cyclosporine nephrotoxicity or recurrent disease.

Clinical data and antidonor HLA antibodies

Clinical information was obtained from hospital charts and laboratory records. Donor and recipient variables as well as transplant and clinical parameters were recorded as shown in Table 1. We studied the impact of HLA mismatches as broad antigens and as cross-reactive groups (CREG) of major histocompatibility complex (MHC) class I [17]. The clinical parameters were obtained at the time of biopsy and at the time of the minimal level of proteinuria following biopsy. Finally, the last measured serum creatinine was collected from all patients with a functioning transplant until death or end of follow-up.

In 13/18 chronic transplant glomerulopathy patients, sera were available at time of biopsy. The presence of circulating antidonor HLA class I and class II antibodies were assessed by enzyme-linked immunosorbent assay (ELISA) and flow cytometer cross-match.

C4d staining

Eleven biopsies from 18 patients with chronic transplant glomerulopathy and three of four biopsies showing acute transplant glomerulitis were available for immunohistochemistry. Fourteen patients with chronic allograft nephropathy without glomerular lesions were randomly selected as control. Polyclonal rabbit anti-C4d antibody (Biomedica, Vienna, Austria), kindly provided by Heinz Regele, was used on paraffin sections as recently described [12, 13]. In brief, 2 μm sections were deparaffinized and endogenous peroxidase activity was blocked with hydrogen peroxide. Antigen retrieval was carried out by pressure-cooking for 10 minutes at 1 bar in citrate buffer (pH 6.0). After overnight incubation with polyclonal anti-C4d antibody (1:250), sections were incubated with horseradish peroxidase (HRP)-conjugated goat antirabbit immunoglobulins absorbed for human IgG. Finally, sections were stained with tyramid-fluorescein isothiocyanate (FITC). Glomerular staining was considered positive if one or more glomeruli showed C4d deposits in the capillary wall. Peritubular capillary staining was scored positive if 25% or more of the peritubular capillaries was strongly positive.

Study design and statistical analysis

Demographic and clinical data of patients with chronic transplant glomerulopathy or chronic allograft nephropathy were compared using the independent samples *t* test for continuous variables and chi-square test for categorical variables. A P value < 0.05 was considered significant. The cumulative incidence of chronic transplant glomerulopathy was determined by the ratio of cases and

Table 1. Patient characteristics of chronic transplant glomerulopathy (CTG) and chronic allograft nephropathy (CAN)

	CTG ($N = 18$)	CAN ($N = 108$)	P value
Recipient factors			
Age *years*	40 ± 11	41 ± 12	0.70
Gender % *female*	39	34	0.71
Cigarette smoking %	47	49	0.91
Peak panel-reactive antibodies %	32 ± 29	29 ± 30	0.72
Current panel-reactive antibodies %	18 ± 30	12 ± 23	0.33
Donor factors			
Age *years*	31 ± 15	41 ± 15	<0.01[a]
Gender % *female*	44	44	0.98
Transplant factors			
Year 1983–1989/1990–1995/1996–2001 %	56/44/0	53/36/11	0.32
Living %	11	8	0.70
Pancreas %	11	6	0.48
Previous %	17	19	0.85
Cold ischemia time *hours*	25 ± 11	26 ± 11	0.79
Delayed graft function %	24	24	0.99
New immunosuppressive agents %	0	8	0.32
HLA CREG mismatches	1.3 ± 1.4	1.9 ± 1.8	0.16
HLA-A-B broad mismatches	1.4 ± 1.0	1.7 ± 1.9	0.51
HLA-DR broad mismatches	0.5 ± 0.7	0.5 ± 0.6	0.95
HLA-A-B-DR broad mismatches	1.9 ± 1.2	2.0 ± 1.5	0.70
Acute rejection episodes			
Type (one/interstitial/vascular) %	33/33/28	23/49/21	0.59
Number (one/two/three or more) %	17/28/22	21/32/29	0.51
First (<2 month/>2 months) %	56/11	56/26	0.21
Last (<3 months/>3 months) %	50/17	34/47	0.04[a]
Characteristic at time of biopsy			
Median time from transplant to biopsy *years*	8.3 (2.6–12.5)	2.0 (0.6–13.7)	<0.01[a]
New immunosuppressive agents %	67	24	<0.01[a]
Serum creatinine *μmol/L*	254 ± 107	247 ± 96	0.78
Creatinine clearance *mL/min*	35 ± 17	37 ± 18	0.66
Serum albumin *g/L*	38 ± 6	40 ± 5	0.09
Serum cholesterol *mmol/L*	5.5 ± 1.1	6.3 ± 1.5	0.03[a]
Proteinuria *g/24 hours*	3.1 ± 3.4	1.8 ± 2.1	0.03[a]
Proteinuria: <0.5/0.5–2.0/>2.0 g/24 hours %	6/44/50	36/35/29	0.03[a]
Systolic blood pressure *mm Hg*	159 ± 17	150 ± 20	0.11
Diastolic blood pressure *mm Hg*	87 ± 10	89 ± 14	0.54
Number of antihypertensive drugs	2.4 ± 1.1	1.8 ± 1.1	0.01[a]
Minimum proteinuria *g/24 hours*	1.4 ± 2.1	1.0 ± 1.6	0.36
Systolic pressure at minimum proteinuria *mm Hg*	144 ± 19	147 ± 21	0.58
Diastolic pressure at minimum proteinuria *mm Hg*	85 ± 11	85 ± 12	0.88

Abbreviations are: HLA, human leukocyte antigen; CREG, cross-reactive group.
[a] P value < 0.05.

total number of patients in the cohort. The time between transplantation and the diagnosis of chronic transplant glomerulopathy and chronic allograft nephropathy was compared using the Kaplan-Meier actuarial method.

To identify the risk factors of chronic transplant glomerulopathy or chronic allograft nephropathy the groups were compared with 739 patients with stable function defined as a last serum creatinine of less than 120% compared to the value at 6 months posttransplantation. The individual effect of the variables on the time to biopsy, graft failure, or end of follow-up was evaluated with the use of the Cox proportional hazard model. Significant predictors ($P < 0.05$) in univariate analysis were fitted into a multivariate model according to a forward selection, likelihood ratio test.

To assess prognostic factors associated with graft failure, outcome was defined as return to dialysis or as a last serum creatinine concentration of more than 150% compared to the value at time of biopsy. Uni- and multivariate Cox regressions were used to evaluate the relationship between the biopsy variables and time between diagnosis and graft failure. Cumulative survival rates were computed by the Kaplan-Meier method.

RESULTS

Clinical features of chronic transplant glomerulopathy and chronic allograft nephropathy

Eighteen patients out of a cohort of 1111 transplants developed chronic transplant glomerulopathy, leading to a cumulative incidence of 1.6%. Chronic allograft nephropathy without transplant glomerulopathy, cyclosporine nephrotoxicity, or recurrent disease was diagnosed in 108 patients (9.7%). Considering these 126 patients together, the percentage of chronic transplant glomerulopathy in chronic allograft nephropathy was 14%. Table 1 shows the clinical data of 18 patients with chronic transplant glomerulopathy in comparison

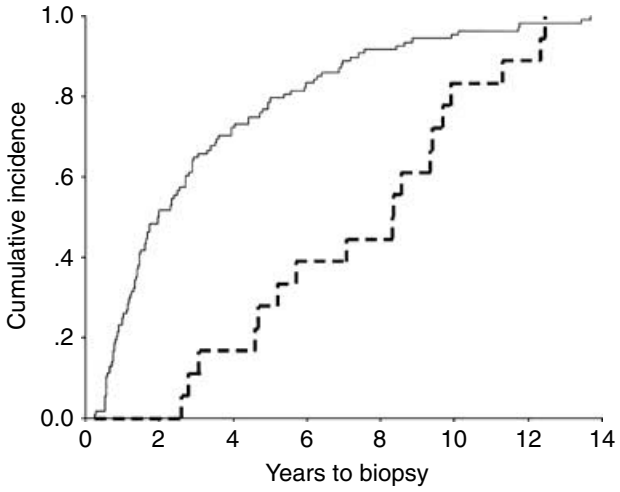

Fig. 1. Cumulative incidence. Chronic transplant glomerulopathy (dashed line, $N = 18$) and chronic allograft nephropathy (solid line, $N = 108$) on follow-up biopsies ($P = 0.004$, log rank test).

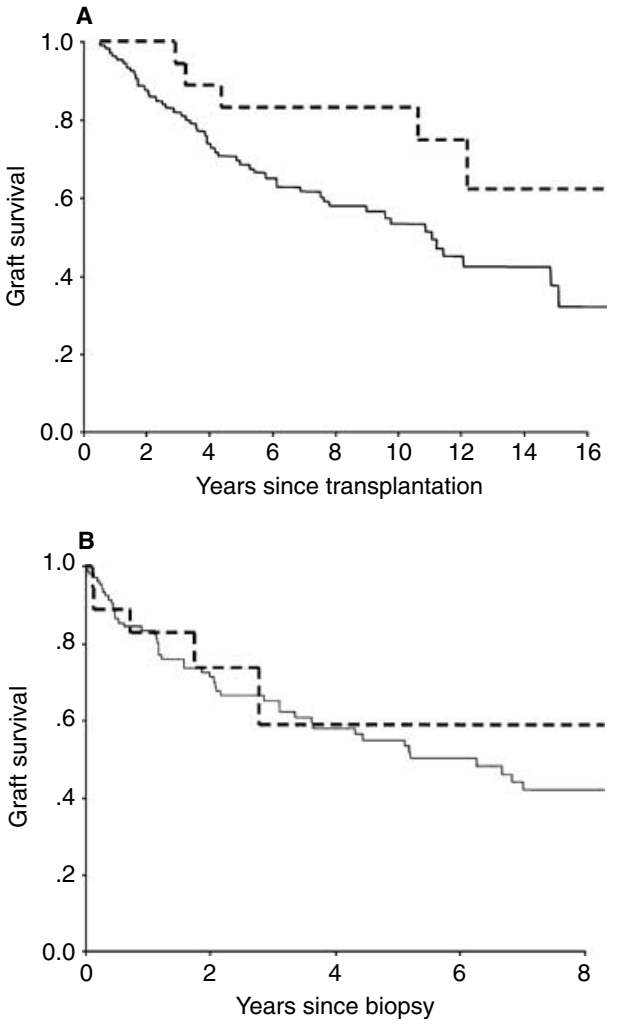

Fig. 2. Graft survival. (*A*) After transplantation of patients with chronic transplant glomerulopathy (dashed line, $N = 18$) and chronic allograft nephropathy (solid line, $N = 108$) ($P = 0.11$, log rank test). (*B*) After diagnosis of chronic transplant glomerulopathy (dashed line, $N = 18$) and chronic allograft nephropathy (solid line, $N = 108$) ($P = 0.99$, log rank test).

with 108 patients with chronic allograft nephropathy. Donor age was significantly lower in the chronic transplant glomerulopathy group. Mean peak and current panel reactive antibodies were not significantly different between the two groups. There was no difference in the number or type of acute rejection episodes but in the chronic transplant glomerulopathy group there were significantly fewer acute rejection episodes beyond 3 months. In comparison with chronic allograft nephropathy, biopsies showing chronic transplant glomerulopathy were obtained later (Fig. 1). At the time of biopsy, the immunosuppressive regimen in the chronic transplant glomerulopathy patients was more often based on Neoral, compared to the chronic allograft nephropathy group. Patients with chronic transplant glomerulopathy had a renal function comparable with chronic allograft nephropathy, but on average more proteinuria. Four out of 18 (22%) chronic transplant glomerulopathy patients had nephrotic syndrome defined by proteinuria of more than 3.5 g per day and an albumin level of less than 35 g/L, in contrast to five out of 111 (4%) cases with chronic allograft nephropathy. Systolic blood pressure was higher in chronic transplant glomerulopathy than in chronic allograft nephropathy despite a higher number of antihypertensive drugs. Graft survival plotted beginning at 6 months after transplantation (Fig. 2A) and at time of biopsy (Fig. 2B) was not significantly different between the two groups.

Characteristics of chronic transplant glomerulopathy

The features and outcome of the patients with chronic transplant glomerulopathy (1 to 18) are shown in Table 2. Renal biopsies were performed because of chronic transplant dysfunction; 16 patients had a decline in renal function defined as an increase in serum creatinine of more than 20% compared to the serum creatinine at 6 months posttransplantation, and 16 had more than 1 g proteinuria per day; hypertension (>140/90 mm Hg) was present in 14 cases, despite antihypertensive medication. Eleven biopsies were adequate and seven were marginal according to the Banff 97 criteria (i.e., all specimens showed at least seven glomeruli and one artery). The diagnosis of chronic transplant glomerulopathy was based on the extent of double contours of the GBM in the most severely affected glomerulus [5], which was present in 10% to 25% of capillary loops in four, 26% to 50% in four and more than 50% in 10 cases (Fig. 3). The degree of proteinuria did not correlate with the level of glomerular damage (Table 2). Seventeen out of 18 cases showed variable increase in mesangial cellularity. Fibrous intimal thickening of arteries was absent in three, mild in seven, moderate in four, and severe in four cases. Chronic allograft nephropathy, based on the extent of interstitial fibrosis

Table 2. Characteristics and outcome of patients with chronic transplant glomerulopathy (CTG) (1 to 18) and acute transplant glomerulitis (ATG) (19 to 22)

		Chronic transplant dysfunction									Follow-up	
							Banff 97 score					
Number	Disease	Time *years*	Creatinine *μmol/L*	Clearance *mL/min*	P *g/24 hours*	BP *mm Hg*	G	CG	CV	CAN	Time *years*	Outcome
1	ADPKD	2.6	234	25	5.2	180/105	3	3	0	2	4.3	Dialysis
2	DM I	2.8	475	16	15.2	175/80	2	2	1	2	2.9	Dialysis
3	GN	3.1	506	22	2.8	160/85	3	3	2	2	3.2	Dialysis
4	CIN	4.6	224	21	3.2	125/55	1	3	1	3	5.6	Decline†
5	ADPKD	4.7	217	31	0.2	180/95	2	1	1	1	10.0	Stable
6	DM I	5.2	406	15	1.0	145/90	2	2	3	1	5.8	Stable
7	Alport	5.7	153	44	3.6	160/100	2	3	2	1	8.0	Decline†
8	CIN	7.1	203	34	1.2	160/90	2	3	1	2	11.2	Stable
9	CIN	8.3	169	49	6.0	155/95	2	2	3	2	17.8	Dialysis
10	GN	8.4	112	85	4.8	160/90	2	3	0	2	12.5	Stable†
11	CIN	8.6	225	18	1.7	190/90	3	2	3	3	8.8	Stable
12	ADPKD	9.4	188	57	1.9	165/85	3	1	1	1	11.4	Stable
13	CIN	9.4	168	48	0.7	160/85	0	3	1	1	12.2	Dialysis
14	GN	9.7	250	41	2.0	140/85	3	3	3	2	10.6	Stable
15	HT	9.9	236	30	2.0	160/85	2	1	2	2	10.6	Dialysis
16	DM I	11.3	270	29	2.7	130/90	2	1	0	2	12.7	Decline
17	CIN	12.3	220	35	1.0	140/85	3	3	1	3	14.9	Stable
18	HT	12.5	313	24	1.2	170/80	3	3	2	2	13.8	Stable
19	DM II	0.6	154	38	11.2	155/95	2	0	3	1	1.6	Dialysis
20	GN	1.2	264	20	2.7	140/100	2	0	3	3	1.5	Dialysis
21	GN	1.9	447	16	7.6	170/95	3	0	3	1	2.1	Dialysis
22	HT	3.3	227	45	2.7	145/95	2	0	1	2	3.9	Dialysis

Abbreviations are: time, time since transplantation; ADPKD, autosomal-dominant polycystic kidney disease; DM I and II, diabetes mellitus type I and type II, respectively; GN, glomerulonephritis; CIN, chronic interstitial nephritis; HT, hypertension; G, glomerulitis; CG, glomerulopathy; CV, fibrous intimal thickening; CAN, chronic allograft nephropathy (grade); BP, blood pressure; P, proteinuria.

†Died.

and tubular atrophy, was graded I, II, and III in five, ten, and three cases, respectively. Immunofluorescence revealed IgM in peripheral capillary loops and mesangial regions in ten patients and weaker reactions to C3, IgG, and IgA in five, four, and three patients, respectively. Circulating antidonor HLA antibodies at the time of biopsy were found in 5/13 patients tested. These antibodies were directed to class I, class II, and class I + II in, respectively, one, three, and one cases. Six patients lost their grafts and returned to dialysis. Three patients had graft failure defined by a creatinine rise of more than 50% at the end of follow-up. Renal function was stable in the other nine patients.

Four patients (19 to 22) had evidence of acute transplant glomerulitis (Table 2). The percentage panel-reactive antibodies at time of transplantation was 17%, 32%, 5%, and 6%, respectively. The patients had either two or three acute rejection episodes. Biopsies showing acute transplant glomerulitis were obtained at 0.6, 1.2, 1.9, and 3.3 years after transplantation. The biopsies showed glomerular infiltration by neutrophils and endocapillary proliferation. Extracapillary proliferation was present in one patient (Fig. 3A). There were no signs of tubulitis or vasculitis, but chronic vascular and tubulointerstitial changes were present in two and four patients, respectively. Antibodies to HLA class I + II of the donor were present in one of the two patients with serum available. Graft loss occurred in all patients, within 1 year after diagnosis.

Risk factors of chronic transplant glomerulopathy and chronic allograft nephropathy

The univariate effects of the various risk factors of chronic transplant glomerulopathy or chronic allograft nephropathy in comparison with patients with a stable function are shown in Table 3. Multivariate Cox regression analysis revealed that current panel-reactive antibodies (RR 1.23, 95% CI 1.05–1.45 per 10% increase, $P = 0.01$) and last acute rejection episodes beyond 3 months (RR 7.6, 95% CI 1.8–31.7, $P = 0.006$) were independently associated with chronic transplant glomerulopathy.

Chronic allograft nephropathy was independently predicted by cigarette smoking (RR 1.80, 95% CI 1.21–2.67, $P = 0.004$), peak panel-reactive antibodies (RR = 1.10, 95% CI 1.03–1.18 per 10% increase, $P = 0.004$), donor age (RR 1.24, 95% CI 1.07–1.43, $P = 0.009$), Neoral and mycophenolate mofetil based regimens (RR 0.46, 95% CI 0.21–0.99, $P = 0.05$), and especially a last acute rejection episode beyond 3 months (RR 14.5, 95% CI 8.3–25.1, $P = 0.0001$).

Glomerular and peritubular C4d staining in transplant glomerulopathy

C4d stained positive in a segmental capillary pattern in the glomeruli of 10/11 chronic transplant glomerulopathy biopsies and in 3/3 biopsies showing acute transplant

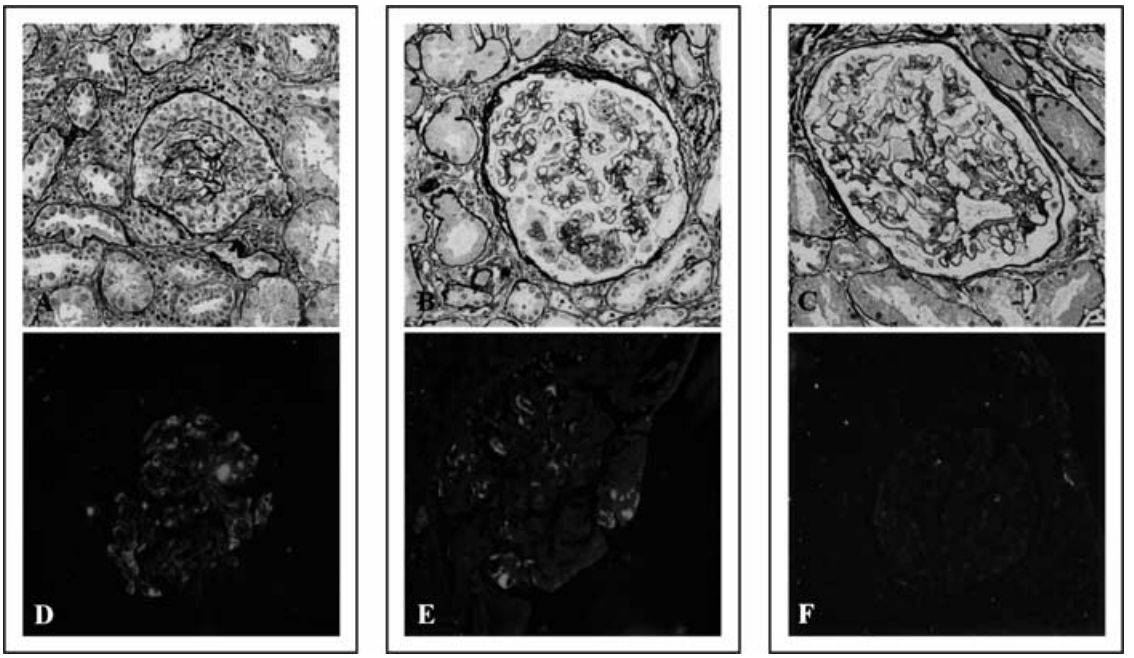

Fig. 3. Light microscopy (*A* to *C*) (silver staining) and immunofluorescence for C4d (*D* to *F*) on paraffin sections. (A and D) Transplant glomerulitis with endo- and extracapillary proliferation and segmental capillary staining of C4d. (B and E) Chronic transplant glomerulopathy with reduplication of the glomerular basement membrane (GBM) and capillary staining of C4d. (C and F) Chronic allograft nephropathy without glomerular lesions and negative C4d staining.

glomerulitis. Figure 3 shows the light and immunofluorescence microscopy of three biopsies with acute transplant glomerulitis, chronic transplant glomerulopathy, and chronic allograft nephropathy without glomerular lesions, respectively. Patient 19 (Fig. 3A, D) had acute transplant glomerulitis, consisting of extensive endo- and extracapillary proliferation at 6 months posttransplantation (Table 2). Patient 11 (B/D) developed chronic transplant glomerulopathy with reduplication of the GBM at $8^1/_2$ years. Panel C/E shows a normal glomerulus without C4d deposits in the biopsy of a patient with chronic rejection at 4 years. Peritubular staining for C4d was also positive in 4/10 chronic transplant glomerulopathy and 3/3 acute transplant glomerulitis cases with glomerular C4d deposits. Antidonor HLA antibodies were detected in 3/10 chronic transplant glomerulopathy and 1/2 acute transplant glomerulitis cases. Glomerular and peritubular C4d deposits were found in one of the 14 control patients with chronic allograft nephropathy ($P < 0.001$ compared to chronic transplant glomerulopathy patients, chi-square test).

Factors predicting graft failure

Table 4 shows factors prognostic for graft failure or loss of renal function in patients with chronic transplant glomerulopathy and chronic allograft nephropathy. Graft failure from chronic transplant glomerulopathy, occurring in nine of the 18 patients, was independently correlated with the lowest level of 24-hour proteinuria (RR = 2.21 per g/day, 95% CI 1.21–4.04, $P = 0.01$). Fifty-one patients in the chronic allograft nephropathy group (46%) lost their graft and 16 (14%) had a rise of serum creatinine of more than 50% at the end of follow-up. Therefore, 67 patients (60%) experienced graft failure. This was predicted by serum creatinine (RR 1.07, 1.04–1.10 per 10 μmol/L, $P < 0.001$) at time of diagnosis, lowest level of proteinuria after biopsy (RR 1.55, 1.24–1.93 per g/day, $P < 0.001$) and the associated systolic blood pressure (RR 1.24, 1.06–1.45 per 10 mm Hg, $P = 0.006$) in multivariate analysis.

DISCUSSION

We studied the clinical and immunohistochemical characteristics of 18 patients with chronic transplant glomerulopathy. The cumulative incidence of chronic transplant glomerulopathy was 1.6%, which is somewhat lower compared to the incidence of 1.9% to 7% reported in other series [1–3]. As biopsies are not invariably obtained in patients with declining graft function, and chronic transplant glomerulopathy has been reported in protocol biopsies the incidence may have been underestimated [18].

Chronic transplant glomerulopathy presents as chronic transplant dysfunction in the cases biopsied, a clinical syndrome consisting of an increased serum creatinine concentration, elevated blood pressure, and proteinuria. Diagnostic biopsies were performed later compared with patients with chronic allograft nephropathy,

Table 3. Univariate analysis of risk factors of chronic transplant glomerulopathy (CTG) and chronic allograft nephropathy (CAN) in comparison with 739 patients with a stable function

	CTG ($N = 18$)		CAN ($N = 108$)	
	RR	95% CI	RR	95% CI
Recipient factors				
Age *10 years*	0.83	0.57–1.23	0.83	0.71–0.97[a]
Gender *female*	1.17	0.45–3.03	1.20	0.81–1.77
Cigarette smoking	0.89	0.21–3.73	1.67	1.14–2.45[a]
Peak panel-reactive antibodies *10%*	1.10	0.96–1.29	1.12	1.06–1.20[a]
Current panel-reactive antibodies *10%*	1.20	1.02–1.40[a]	1.14	1.04–1.23[a]
Donor factors				
Age *10 years*	0.77	0.53–1.12	1.26	1.10–1.44[a]
Gender *female*	0.87	0.34–2.19	0.95	0.65–1.38
Transplant factors				
Year 1990–1995 vs. 1983–1989	1.52	0.53–4.32	0.62	0.41–0.95[a]
Year 1996–2001 vs. 1983–1989	NA	NA	0.37	0.20–0.70[a]
Living	0.66	0.15–2.88	0.52	0.26–1.02
Pancreas	2.50	0.56–11.3	0.70	0.33–1.51
Previous	1.13	0.33–3.91	1.43	0.88–2.32
Cold ischemic time *hour*	1.01	0.97–1.05	1.02	1.00–1.04[a]
Delayed graft function %	1.08	0.35–3.32	1.18	0.75–1.84
New immunosuppressive agents	0.04	0.00–85.3	0.30	0.15–0.63[a]
HLA CREG mismatches	0.93	0.66–1.30	1.12	1.00–1.26[a]
HLA-A-B mismatches	0.95	0.60–1.50	1.06	0.93–1.20
HLA-DR mismatches	1.70	0.54–2.52	0.95	0.69–1.30
HLA-A-B-DR mismatches	1.00	0.70–1.43	0.98	0.86–1.12
Acute rejection episodes				
Interstitial	8.21	0.91–74.3	3.59	2.32–5.77[a]
Vascular	3.36	1.02–10.9[a]	3.60	2.04–6.34[a]
Number	1.63	1.07–2.98[a]	1.87	1.58–2.13[a]
First episode within 2 months	7.93	0.97–64.7	3.06	1.85–5.07[a]
First episode after 2 months	10.7	0.66–171	13.5	7.57–23.9[a]
Last episode within 3 months	7.34	0.88–61.2	1.97	1.15–3.39[a]
Last episode after 3 months	12.6	1.13–139[a]	16.4	9.72–27.6[a]

Abbreviations are: HLA, human leukocyte antigen; CREG, cross-reactive group; NA, not applicable.
[a] P value < 0.05.

confirming that the clinical manifestations of chronic transplant glomerulopathy tend to develop late after transplantation [3]. At the time of diagnosis, patients with chronic transplant glomerulopathy had a similar degree of renal dysfunction, but on average the serum albumin level was lower and proteinuria more severe than patients with chronic allograft nephropathy, compatible with glomerular injury. We documented an increased systolic blood pressure and use of antihypertensive drugs in patients with chronic transplant glomerulopathy. However, more chronic transplant glomerulopathy patients used cyclosporine (Neoral) at time of biopsy, an agent with increased bioavailability that accounted for increased blood pressure and proteinuria after conversion from Sandimmune in the Leiden cohort of renal transplants [14].

Double GBM contours are a key light microscopic feature of chronic transplant glomerulopathy and were required for the diagnosis of chronic transplant glomerulopathy in our study. We observed in all but one patient a variable degree of glomerulitis in the glomerular tuft, a feature characteristic of acute transplant glomerulitis [4, 16]. We confirm earlier results from our center and from others that chronic vascular changes did not parallel chronic transplant glomerulopathy [3, 19]. Ten patients showed no or minor obliteration of the arterial lumen, indicating that ischemia is not a likely mechanism responsible for chronic transplant glomerulopathy. The observed chronic tubulointerstitial changes are most likely related to previous acute rejection episodes [20].

Late acute transplant glomerulitis, observed in four patients, occurred earlier than chronic transplant glomerulopathy and was associated with severe proteinuria and subsequent rapid graft failure. Messias et al [16] examined early glomerulitis, that occurred in 28/63 (44%) patients with acute rejection within 3 months. Due to a strong association with vascular rejection there was no independent effect on graft survival [16]. In our opinion, early glomerulitis at time of an acute rejection episode should be distinguished from late onset transplant glomerulitis.

Assessment of risk factors revealed that both chronic transplant glomerulopathy and chronic allograft nephropathy were strongly related with pretransplant sensitization and late acute rejection episodes, suggesting chronic rejection. These factors were also present in patients with acute transplant glomerulitis. In the study of Messias et al [16], a significantly higher percentage of patients in the early glomerulitis group was highly

Table 4. Prognostic factors of graft failure from chronic transplant glomerulopathy (CTG) and chronic allograft nephropathy (CAN)

	CTG ($N = 18$)		CAN ($N = 108$)	
	RR	95% CI	RR	95% CI
Time since transplantation *years*	0.71	0.51–0.98[a]	0.97	0.89–1.07
New immunosuppressive agents	0.48	0.12–1.94	1.07	0.51–2.22
Serum creatinine *10 μmol/L*	1.15	1.04–1.29[a]	1.05	1.03–1.08[a]
Endogenous creatinine clearance *10 mL/min*	0.41	0.16–1.06	0.74	0.61–0.89[a]
Serum albumin *g/L*	0.82	0.70–0.97[a]	0.94	0.89–0.99[a]
Proteinuria *g/24 hours*	1.29	1.01–1.64[a]	1.32	1.19–1.48[a]
Minimum proteinuria *g/24 hours*	1.45	1.08–1.95[a]	1.77	1.46–2.15[a]
Systolic blood pressure *10 mm Hg*	1.08	0.63–1.88	1.15	1.01–1.29[a]
Systolic blood pressure at minimum proteinuria *10 mm Hg*	1.24	0.84–1.82	1.40	1.23–1.60[a]
Diastolic blood pressure *10 mm Hg*	0.67	0.36–1.27	1.17	1.01–1.34[a]
Diastolic blood pressure at mininum proteinuria *10 mm Hg*	2.14	0.94–4.89	1.38	1.08–1.77[a]

[a] P value < 0.05.

sensitized pretransplantation, had retransplants and had delayed graft function compared to the nonglomerulitis group. Preformed anti-HLA antibodies are related to acute humoral rejection and also increase the risk of chronic allograft nephropathy [21, 22]. In comparison with transplants with stable function both chronic transplant glomerulopathy and chronic allograft nephropathy were associated with late acute rejection episodes. We found earlier that acute rejection episodes beyond 3 months have a detrimental impact on long-term outcome and are associated with CREG mismatches [17, 23]. At this time posttransplantation, indirect allorecognition (i.e., activation of T helper cells by donor MHC molecules presented by recipient antigen presenting cells) may trigger the production of antibodies that may mediate chronic rejection [11, 24]. Therefore, both preexisting and newly formed antibodies posttransplantation may increase the risk of chronic allograft nephropathy and chronic transplant glomerulopathy.

Because of this risk profile of chronic transplant glomerulopathy, we decided to investigate C4d deposition in the transplants. We used a polyclonal anti-C4d antibody suitable for detection of glomerular C4d on paraffin sections [12, 13]. Glomerular deposits of C4d were present in 10/11 biopsies with chronic transplant glomerulopathy and in 3/3 cases showing acute transplant glomerulitis. Peritubular staining for C4d was positive in respectively 4/10 and 3/3 cases with glomerular C4d deposits. Absence of glomerular C4d staining in all but one tested patients with chronic allograft nephropathy suggest that this antibody might be useful in characterizing late transplant glomerulopathy. Endothelial deposition of the complement split product C4d in peritubular capillaries has been established as a marker for both acute and chronic humoral rejection defined by the presence of antidonor HLA antibodies [9]. Absence of concomitant capillary immunoglobulin staining in C4d-positive biopsies has been explained by less covalent binding compared to C4d [9]. Recently, C4d deposition in peritubular capillaries was detected on paraffin sections in 34% of 213 late biopsies and found to be associated with tubular basement membrane multilayering and chronic transplant glomerulopathy [12]. However, in contrast to our data, glomerular C4d staining was observed in only 12% of the chronic transplant glomerulopathy biopsies, which is difficult to explain unless their criteria for the diagnosis of chronic transplant glomerulopathy were less strict [12]. In biopsies with unaffected glomeruli but positive C4d in peritubular capillaries, progression to chronic transplant glomerulopathy could be observed in follow-up biopsies [12]. Ongoing humoral rejection may link early glomerulitis and late transplant glomerulopathy. However the acute transplant glomerulitis cases presented in this study were all rapidly progressive and did not progress into chronic transplant glomerulopathy suggesting that acute transplant glomerulitis not necessarily results in chronic transplant glomerulopathy.

The evidence for humoral rejection in late transplant glomerulopathy suggests that antibodies directed against donor HLA antigens play a role. In a series of chronic humoral rejection, 15 out of 17 patients with C4d in the peritubular capillaries had antidonor HLA antibodies [11]. We could detect antidonor HLA antibodies in 3/10 chronic transplant glomerulopathy cases with glomerular C4d deposits, suggesting that a tissue-specific response might also be involved. However, we cannot exclude that the levels of circulating anti-HLA antibodies are undetectable due to absorption by antigens in the graft. In an experimental model, we found circulating and kidney graft bound IgG antibodies against the GBM in rats with chronic transplant glomerulopathy. Using proteomic techniques the heparan sulfate proteoglycan perlecan and the α1 chain of collagen VI in association with the α5 chain of collagen IV were identified as the antigens recognized by the antibodies [25]. We hypothesize that similar responses against glomerular antigens are also present in patients with transplant glomerulopathy.

Humoral rejection warrants a specific therapeutic strategy. In chronic rejection decrease of antidonor HLA antibodies and C4d deposition can be induced by rescue

therapy with tacrolimus and mycophenolate mofetil [26]. Furthermore, patients who stay on mycophenolate for a prolonged period of time have a lower risk of late acute rejection episodes and chronic allograft nephropathy [27, 28]. In our series, only one patient with chronic transplant glomerulopathy, remarkably the subject without C4d deposits, used mycophenolate mofetil at time of diagnosis. As this agent was only introduced in our center in 1997, longer follow-up is needed to determine whether this agent might reduce the incidence of chronic transplant glomerulopathy as well.

The prognosis of chronic transplant glomerulopathy was related to the time of diagnosis and level of proteinuria. However, once the diagnosis has been made we found a similar graft survival rate compared to patients with earlier diagnosed chronic allograft nephropathy. In both groups outcome correlated with proteinuria, in the chronic allograft nephropathy group together with concomitant systolic blood pressure and renal function at time of diagnosis. These results support recent evidence that renoprotection could be achieved when long-lasting angiotensin-converting enzyme (ACE) inhibition results in persistent reduction in proteinuria [29, 30]. ACE inhibitors and angiotensin II antagonists are well tolerated in transplant recipients with chronic allograft nephropathy and are associated with stabilization of renal function [31, 32].

CONCLUSION

Chronic transplant glomerulopathy may present years after transplantation. Sensitization and late acute rejection episodes were identified as risk factors strongly suggesting an underlying immunologic mechanism. The presence of glomerular C4d deposits supports a role for humoral immune responses in the pathogenesis of chronic transplant glomerulopathy.

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Reprint requests to Yvo Sijpkens, Department of Nephrology, C3-P, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands.
E-mail: y.w.j.sijpkens@lumc.nl

REFERENCES

1. Maryniak RK, First MR, Weiss MA: Transplant glomerulopathy: Evolution of morphologically distinct changes. *Kidney Int* 27:799–806, 1985
2. Habib R, Zurowska A, Hinglais N, *et al*: A specific glomerular lesion of the graft: Allograft glomerulopathy. *Kidney Int* 44(Suppl 42):S104–S111, 1993
3. Suri DL, Tomlanovich SJ, Olson JL, Meyer TW: Transplant glomerulopathy as a cause of late graft loss. *Am J Kidney Dis* 35:674–680, 2000
4. Racusen LC, Solez K, Colvin RB, *et al*: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 55:713–723, 1999
5. Halloran PF: Call for revolution: A new approach to describing allograft deterioration. *Am J Transplant* 2:195–200, 2002
6. Andresdottir MB, Assmann KJ, Koene RA, Wetzels JF: Immunohistological and ultrastructural differences between recurrent type I membranoproliferative glomerulonephritis and chronic transplant glomerulopathy. *Am J Kidney Dis* 32:582–588, 1998
7. Gough J, Yilmaz A, Miskulin D, *et al*: Peritubular capillary basement membrane reduplication in allografts and native kidney disease: A clinicopathologic study of 278 consecutive renal specimens. *Transplantation* 71:1390–1393, 2001
8. Habib R, Broyer M: Clinical significance of allograft glomerulopathy. *Kidney Int* 44 (Suppl 43):S95–S98, 1993
9. Mauiyyedi S, Colvin RB: Humoral rejection in kidney transplantation: New concepts in diagnosis and treatment. *Curr Opin Nephrol Hypertens* 11:609–618, 2002
10. Racusen L, Colvin R, Solez K, *et al*: Antibody-mediated rejection criteria—An addition to the Banff '97 classification of renal allograft rejection. *Am J Transplant* 3:708–714, 2003
11. Mauiyyedi S, Pelle PD, Saidman S, *et al*: Chronic humoral rejection: Identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 12:574–582, 2001
12. Regele H, Bohmig GA, Habicht A, *et al*: Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: A contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 13:2371–2380, 2002
13. Regele H, Exner M, Watschinger B, *et al*: Endothelial C4d deposition is associated with inferior kidney allograft outcome independently of cellular rejection. *Nephrol Dial Transplant* 16:2058–2066. 2001
14. Sijpkens YW, Mallat MJ, Siegert CE, *et al*: Risk factors of cyclosporine nephrotoxicity after conversion from Sandimmune to Neoral. *Clin Nephrol* 55:149–155, 2001
15. Cruzado JM, Carrera M, Torras J, Grinyo JM: Hepatitis C virus infection and de novo glomerular lesions in renal allografts. *Am J Transplant* 1:171–178, 2001
16. Messias NC, Eustace JA, Zachary AA, *et al*: Cohort study of the prognostic significance of acute transplant glomerulitis in acutely rejecting renal allografts. *Transplantation* 72:655–660, 2001
17. Sijpkens YW, Doxiadis II, de Fijter JW, *et al*: Sharing cross-reactive groups of MHC class I improves long-term graft survival. *Kidney Int* 56:1920–1927, 1999
18. Furness PN, Taub N: International variation in the interpretation of renal transplant biopsies: Report of the CERTPAP Project. *Kidney Int* 60:1998–2012, 2001
19. Sijpkens YWJ, Doxiadis IIN, van Kemenade FJ, *et al*: Chronic rejection with or without transplant vasculopathy. *Clin Transplant* 17:163–170. 2003
20. Bonsib SM, Abul-Ezz SR, Ahmad I, *et al*: Acute rejection-associated tubular basement membrane defects and chronic allograft nephropathy. *Kidney Int* 58:2206–2214, 2000
21. Crespo M, Pascual M, Tolkoff-Rubin N, *et al*: Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics. *Transplantation* 71:652–658, 2001
22. Takemoto SK, Cho YW, Gjertson DW: Transplant risks. *Clin Transpl* 325–334, 1999
23. Sijpkens YW, Doxiadis II, Mallat MJ, *et al*: Early versus late acute rejection episodes in renal transplantation. *Transplantation* 75:204–208, 2003
24. Sayegh MH: Why do we reject a graft? Role of indirect allorecognition in graft rejection. *Kidney Int* 56:1967–1979, 1999
25. Joosten SA, van Dixhoorn MG, Borrias MC, *et al*: Antibody response against perlecan and collagen types IV and VI in chronic renal allograft rejection in the rat. *Am J Pathol* 160:1301–1310, 2002
26. Theruvath TP, Saidman SL, Mauiyyedi S, *et al*: Control of antidonor antibody production with tacrolimus and mycophenolate

mofetil in renal allograft recipients with chronic rejection. *Transplantation* 72:77–83, 2001
27. Ojo AO, Meier-Kriesche HU, Hanson JA, *et al*: Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. *Transplantation* 69:2405–2409, 2000
28. Meier-Kriesche HU, Steffen BJ, Hochberg AM, *et al*: Long-term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. *Am J Transplant* 3:68–73, 2003
29. Shiigai T, Shichiri M: Late escape from the antiproteinuric effect of ace inhibitors in nondiabetic renal disease. *Am J Kidney Dis* 37:477–483, 2001
30. Ruggenenti P, Perna A, Remuzzi G: ACE inhibitors to prevent end-stage renal disease: when to start and why possibly never to stop: A post hoc analysis of the REIN trial results. Ramipril efficacy in nephropathy. *J Am Soc Nephrol* 12:2832–2837, 2001
31. Holgado R, Anaya F, Del Castillo D: Angiotensin II type 1 (AT1) receptor antagonists in the treatment of hypertension after renal transplantation. *Nephrol Dial Transplant* 16 (Suppl 1):117–120, 2001
32. Lin J, Valeri AM, Markowitz GS, *et al*: Angiotensin converting enzyme inhibition in chronic allograft nephropathy. *Transplantation* 73:783–788, 2002